DEPARTMENT OF PHARMACY AND CHEMISTRY.

Edited by FRED I. LACKENBACH.

Serums and Vaccines of the U. S. P. and N. N. R.

1. (First of Series.)

The universally acknowledged "specific" in the prophylaxis and treatment of diphtheria, no product is more worthy of first mention in this series than is diphtheria antitoxin.

Serum Antidiphthericum is official in the United States and German Pharmacopeias and is described in the U. S. P. as "A fluid separated from the coagulated blood of a horse immunized through the inoculation of diphtheric toxin."

The initial process in the preparation of antitoxin is the securing of a pure culture of diphtheria bacilli from a throat infected with the disease. A pledget of sterilized cotton mounted on a swab is applied to the diseased tissue and smeared on a slant of Loeffler's blood-serum media contained in a test tube. This is placed in an incubator kept at the body temperature for twelve or more hours when numerous, roundish pin-point colonies will have formed upon the surface. Among these will be found pure cultures of the Klebs-Loeffler diphtheria bacillus. These in turn are transferred by means of a sterilized platinum wire to fresh tubes of blood-serum and again incubated. From these pure cultures other tubes are planted which serve to inoculate large flasks of specially prepared beef bouillon. The planted flasks are now placed in an incubator, where in the course of several days countless millions of diphtheria germs are grown, giving rise to large quantities of diphtheria toxine. Trikresol is added to kill the germs and the culture is filtered through unglazed porcelain to remove the bacteria. The clear filtrate contains the soluble products elaborated by the growing and multiplying germs. This extremely virulent poison is called diphtheria toxin. This toxin is standardized by inoculating guinea-pigs of standard weight with graduated quantities of toxin. The smallest quantity proving fatal to the guinea-pig within (usually) four days, is employed as the basis of dosage in inoculating the larger animals.

Perfectly sound horses are injected subcutaneously with increasing quantities of the toxin, beginning with one or more lethal guinea-pig doses and increasing as the animal acquires immunity, to from 75,000 to 125,000 fatal guinea-pig doses, in volume approximating 0.1 to 250. to 500. cc. of the toxin. The injections are given at intervals of a few days and continue over several months, until the height of the animal's immunity is reached. As the animal develops immunity to the toxin, **antitoxin** is formed. This antitoxin is a reaction product of the living organism. The body cells are attacked by the poison, and if not destroyed, are stimulated in the overproduction of "antibodies" capable of combining with and neutralizing the poison (Ehrlich).

The horse is allowed to rest during a week or two and a preliminary test is made of the antitoxic strength of his blood-serum. If this comes up to requirements the animal is bled by passing a canula attached to a sterilized rubber tube, into the external jugular vein. From five to ten liters of blood is drawn off into large test tubes which are set aside to clot. The serum separated from the clot, filtered, and with an added preservative, is the **Diphtheria Antitoxin** of the market.

The physiological activity of Antitoxin is determined by the number of immunity units contained in each cc. This may vary from 200 units in poor serum, to 1500 units per cc. in high-potency serum. The quality (as also the cost of production) is determined by the number of units contained in a given volume of the serum. The immunity unit is a measure of antitoxic power—not of quantity or volume. It is an arbitrary quantity based upon physiological test, and depends upon the neutralization of toxin by antitoxin in the body of the guinea-pig, which animal is highly susceptible to the diphtheria bacillus and its poisons.

Under the Act of Congress approved July 1, 1902, all Diphtheria Antitoxin sold in the United States is required to conform to the standard established by the Public Health and Marine Hospital Service. This standard is based on the Ehrlich Immunity Unit preserved at the Royal Institute for Experimental Therapy at Frankfort-on-the-Main. Antitoxins of foreign production are standardized and sealed in government laboratories before they are marketed, but in the United States antitoxins are tested in comparison with the Government standard unit in the laboratory of each individual producer. This standard unit is prepared and preserved with the most exacting care at the Hygienic Laboratory, Washington, D. C. At intervals of two months about 10 cc. of the standard unit serum is distributed to each of the licensed manufacturers. This is a glycerin solution of dried antitoxin, and properly diluted contains one antitoxic unit in each cc. The standard antitoxic unit is used to standardize a laboratory test toxin which determines that amount (approximately 100 fatal guinea-pig doses), which just equals or neutralizes the unit when the two are mixed together and injected into a 250 gm. (standard weight) guinea-pig, the life or death of the guinea-pig within a period of four days serving as indicator. The strength of all unknown antitoxins is tested against this standardized test toxin.

Globulin Antitoxin (Antidiphtheric Globulins)—"Concentrated Diphtheria Antitoxin" represents in a concentrated form the antitoxic elements of the natural serum.

For many years attempts were made to concentrate diphtheria antitoxin. First, the amount of water in the serum was reduced by subjecting the serum to a freezing temperature and removing the ice particles. Then, the evaporation of a portion of the water in vacuo was tried. These proving unavailing, every effort was made to increase the immunity of the horse, to increase the potency of the serum, but there proved a limit to the horse's immunity. Finally, in attempting to isolate the antitoxin from the non-essential elements of the serum, scientists discovered the antitoxic principle to be a globulin, or possessed of such properties that it was precipitated with the globulins. Further, it was demonstrated that the quantitative amount of globulin in the serum of immunized horses increased as the antitoxic strength of their blood increased.

After many processes were elaborated for the separation of the antitoxic globulin from the serum, the following process perfected by Gibson, is the one generally adopted:

A quantity of antitoxic serum is added to an equal volume of a saturated solution of ammonium sulphate. A heavy flocculent, waxy precipitate of the serum globulins results which is separated from the serum-albumin, nucleo-proteids and other inert substances by filtration. The precipitate, containing the antitoxin of the serum, is added to a saturated solution of sodium chloride in which the antitoxic- or pseudo-globulin, goes into solution leaving behind the insoluble euglobulins. These are separated by filtration, the filtrate containing the antitoxin of the serum taken. The antitoxic globulin is then precipitated from the salt solution by the addition of acetic acid. The resulting heavy flocculent precipitate is separated by filtration and dried between layers of absorbent filter paper. The white, waxy mass is then placed in a bag of dialyzing parchment and dialyzed in running water for several days during which the mass gradually liquefies to a fluid resembling the original serum. This is neutralized with sodium hydroxide and the dialysis continued until it is freed from all adhering salts, etc. This fluid is from one-half to one-third less the original volume of the serum and contains nearly all the antitoxin. Sodium chloride then restores the normal

salt content and a preservative is added. Finally, the globulin-antitoxin is filtered through paper, then through a Berkefeld filter, and tested in the same manner as is the regular (U. S. P.) antitoxin.

The product still further concentrated and dried in vacuo, is the **Dried Antitoxin Globulin.** This is intended for the extemporaneous preparation of the fluid antitoxin by dissolving in sterile distilled water. It occurs in 3000- and 5000-unit packages and is useful in emergencies where the natural serum is unobtainable. It contains no preservative and keeps indefinitely.

Many conflicting statements have been made for, and against, the globulin form of antitoxin, the chief arguments in favor being its greater concentration and lessened liability to produce urticaria. On the other hand, the intricate chemical processes involved in its preparation may be destructive to delicate bactericidal properties contained in normal serum. It would seem from the consensus of opinion, and taking into account the high-potency natural serum now on the market, that for large doses—upward of 5000 units and over, the "concentrated" (globulin) form is to be preferred. For smaller dosage, the natural (U. S. P.) serum should fulfill requirements. Since there is economy in extracting the globulins from low-grade, discarded, and out-of-date serum, which would otherwise be a loss to the manufacturer, it would be well for physicians to weigh carefully the respective merits of the two forms in their own clinical experience. Physicians should indicate on their orders whether U. S. P. or Globulin serum is desired.

Antidiphtheric Serum, both U. S. P. and Globulin, appears on the market in bulbs, vials, and most generally in piston-syringe containers, in packages containing 500, 1000, 2000, 3000, 4000, 5000 and up to 10,000 units. The serum gradually loses in power, the loss in one year varying between ten and thirty per cent. The date beyond which the serum will no longer have the strength indicated, appears on the label, but manufacturers generally allow an excess of units so that serum not too long out-of-date can be relied upon in case no fresh supply is available.

The U. S. P. gives 3000 units as the average dose, and 500 units as immunizing dose for well persons. As the main problem presented in a case of diphtheria is the neutralization of a specific toxin, the antidote cannot be too soon administered, and in doses sufficient to neutralize the poison beyond the shadow of a doubt. An excess of antitoxin can do no harm, while in laboratory experiments on guinea-pigs, it is shown that the delay of only one hour after the injection of diphtheria toxin, makes necessary the administration of forty times as much antitoxin as would be necessary with simultaneous injections of toxin and antitoxin. A "given up" case of diphtheria recovered after the use of 160,000 units. Detailed information as to dosage and mode of administration invariably accompanies the package.

The Act of Congress approved July 1, 1902, provides that no one be allowed to engage in interstate traffic in antitoxin without a license issued by the Secretary of the Treasury on recommendation of the Surgeon-General of the Public Health and Marine Hospital Service. This license is issued only after a careful inspection of the establishment, its methods of manufacture, and an examination of its products for purity and potency. It regulates also the sale of viruses, serums, toxins and analogous products, and imposes upon the Director of the Hygienic Laboratory the duty of examining such products. From time to time purchases are made on the open market by officers of the P. H. and M. H. S. stationed in various parts of the country and the products sent to the Hygienic Laboratory where they are examined for potency and freedom from contamination by foreign bacteria and chemical poisons, especially tetanus toxin. If found not to conform to the prescribed requirements, the manufacturer is notified to withdraw that particular lot from sale and guard against a repetition of the offense.

REFERENCES:

United States Pharmacopeia—8th Revision (1905).
The National-Standard Dispensatory.
The Immunity Unit—Bulletin No. 21, P. H. and M. H. S.
New and Non-official Remedies, A. M. A.
Bulletins: The Cutter Laboratory, Berkeley, Cal.
H. K. Mulford Co., Philadelphia, Pa.
Parke, Davis & Co., Detroit, Michigan.

PROCEEDINGS OF THE SAN FRANCISCO COUNTY MEDICAL SOCIETY.

During the month of February the following meetings were held:

Section on Medicine, Tuesday, February 7, 1911.

1—Presentation of a Case of Polycythemia with Splenomegaly, and a Case of Alkaptonuria with Pigmentation of Skin and Cartilages, Major P. M. Ashburn, United States Army.

2—Exhibition of Cases of Pituitary Disease, Herbert C. Moffitt. Discussed by Drs. Quinan and Moffitt.

3—Use and Abuse of Tuberculin, Wm. C. Voorsanger.

General Meeting, Tuesday, February 14, 1911.

1—Discussion on paper "Vaccine Therapy" by A. F. Shafer, read at the January meeting. Drs. Rosenstirn, Dannenbaum, N. N. Brown, Kuhlman, Cheney, Arnold, P. K. Brown, Power, Bine, Russ, Clark, Tait, Hunkin, Quinan, Ryfkogel, Porter, Artigues and Coffey.

2—Bovine Tuberculosis in its Relation to Public Health, Geo. S. Baker, U. S. Dept. Agriculture. Discussed by Drs. Rosenstirn, Fleischner, Chipman, Porter, Kuhlman, Baker.

Section on Surgery, Tuesday, February 21, 1911.

1—Presentation of Case, E. G. Frisbie.

2—Demonstration of Two Specimens of Large Vesical Calculi Removed from Female Bladders by Litholapaxy and the Operating Cystoscope, Henry Meyer.

3—A Report of Four Cases of Perforating Gunshot Wounds of the Abdomen, I. W. Thorne.

4—A Gauze Sponge Left in the Skull for Over Six and One-half Years, Harry M. Sherman. Discussed by Drs. Rosenstirn and Sherman.

5—Two Cases of Acute Perforating Diverticulitis, Chas. G. Levison. Discussed by Drs. Russ, Eloesser, Rosenstirn, Sherman, Levison.

Eye, Ear, Nose and Throat Section, Tuesday, February 28, 1911.

1—Demonstration of Cases, V. F. Lucchetti. Discussed by Dr. Welty.

2—Demonstration of Cases of Tuberculosis of the Eye, E. W. Alexander.

3—Report of Recent Ear Literature, Harrington B. Graham.

4—Report of Recent Eye Literature, E. W. Alexander.

5—The Eye Symptoms of Intracranial Growth, Wm. F. Blake. Discussed by Drs. Pischel, Alexander, McClenahan, Welty, Pischel, Blake.

Section on Medicine, February 7, 1911.

Exhibition of Cases of Pituitary Disease.

By HERBERT C. MOFFITT, M. D., San Francisco.

In the California State Journal of July of last year I reported several cases of hypophysis disease. This evening I desire to present patients illustrating both hypo- and hyper-pituitarism.

Case 1. The first patient is a woman of 29, unmarried, born in San Francisco. She was always a fat baby and child but not abnormally so. At the age of 11 she had severe scarlet fever and her mother says some abscesses formed "between the nose and the mouth." This suggests she may have had some suppuration in the pharynx or possibly in the nasal sinuses. She began to have some headache at the age of 12 or 13 and grew considerably